Methods: In the present study, we have identified and characterized the expression of *Anopheles culicifacies* nitric oxide synthase (AcNOS) in non refractory (species A) and refractory (species B) in order to elucidate a plausible mechanism of refractoriness in terms of NOS physiologies. Understanding the difference between vector and non-vector mosquitoes can facilitate development of novel malaria control strategies

Results: The specific activity of AcNOS and circulating levels of nitrite/nitrate in mid-guts and haemolymph, end products of NO synthesis, were found to be significantly higher in the refractory species B as compared to non-refractory species A soon after invasion of the midgut by *Plasmodium vivax* at the beginning and during the course of blood feeding. Dietary feeding of a NOS inhibitor, L-NAME significantly decreased AcNOS enzyme activity and increased the parasite numbers (oocysts) in infected mosquitoes, confirming that *An. culicifaces* species B limits *Plasmodium* development via a NO mechanism. Amplification of the AcNOS gene fragment (200 bp) and sequence analysis show the highest level of homology to other characterized inducible NOS genes. Increased levels of mRNAs (encoding iNOS) were observed at 7 days and 9-14 days after ingestion of an infected blood meal using semiquantitative RT-PCR analyses in the refractory species. Nitric oxide synthase (NOS) gene elements inhibitory to growth of malaria parasite *in vitro* and *An. culicifaces NOS* gene (AcNOS) is transcriptionally activated by the malaria parasite *Plasmodium vivax* in refractory mosquitoes

Conclusion: Our studies have revealed that AcNOS may be used as an additional effector gene to block the development of the malaria parasite in *An. culicifaces* mosquitoes. Our studies are important for understanding of innate immune-related anti-parasite defenses of the mosquito, parasite-vector interactions and may relate to/elucidate the mechanism of refractoriness and fight against the disease.

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29.012

Malaria vector studies in the Republic of Korea: Vector parasite rates and habitat distribution

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Background: In 1993, vivax malaria reemerged along the demilitarized zone (DMZ) of the Republic of Korea (ROK) and rapidly increased to more than 4,000 cases by 2000. Although it was presumed that malaria would rapidly spread throughout Korea, malaria transmission remained concentrated near the DMZ. In 2005, two new species of Anopheles mosquitoes were identified, with studies indicating that Anopheles pullus and An. kleini were likely the primary vectors, while An. sinensis s.s., was a secondary vector. New An. kleini, and An. belenrae are found throughout Korea, population densities are highest near the DMZ and possibly accounting for the high rates of transmission in this area.

Methods: More than 5,000 larvae were collected from selected habitats near Warrior Base (approximately 3 Km south of the DMZ), labeled, placed in 100% ethanol, and shipped to the Walter Reed Biosystematics Unit where they were identified by PCR to species. Additionally, >7,000 adult anopheline mosquitoes were collected by light traps, Mosquito Magnets, and resting collections at selected sites in northern Gyeonggi and Gangwon Provinces (1-30 Km south of the DMZ). The head and thorax of individual specimens were identified to species by PCR and sporozoites, and malaria infected mosquitoes identified by single step and semi-nested multiplex-PCR.

Results: Larvae were identified to species from selected habitats and include Anopheles sinensis s.s., An. pullus, An. kleini, An. belenrae, An. lesteri, and An. sineroides. Rice paddies were the predominant habitat sampled. From adult collections, Plasmodium vivax was identified in An. belenrae, An. kleini, An. pullus, and An. sinensis s.s. We discuss the potential role of these vector species in maintaining malaria in the ROK.

Conclusion: The identification of potential malaria vectors, their role in malaria transmission, and their distributions, including population density, are important in understanding the dynamics of transmission and epidemiology of human cases in the ROK. Studies to determine the distributions of Anopheles spp. and their relative population densities over their range are needed.

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29.013

Cardiac function and haemodynamics in African children with severe malaria

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Background: Mortality from severe malaria remains unacceptably high in sub-Saharan Africa. Several markers of cardiovascular compromise and metabolic acidosis correlate with mortality. The role of cardiac dysfunction in the pathogenesis of severe childhood malaria remains unknown.

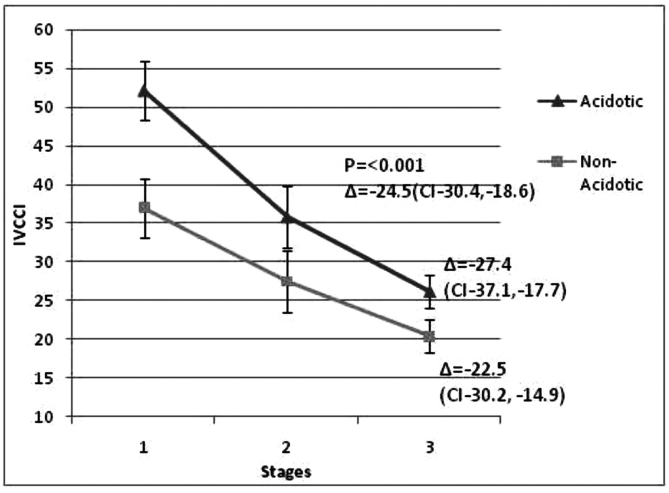
In this study we aimed to investigate cardiac function and haemodynamic status of children admitted with severe malaria, assessing changes over time and response to fluid resuscitation.

Methods: Setting:High Dependency Unit, Kilifi District Hospital, Kenya. We examined thirty children admitted with severe malaria using portable echocardiography to assess their cardiac function and haemodynamic status on admission (day 0), day 1, and discharge. We compared

haemodynamic parameters in two study groups: children presenting with metabolic acidosis (base deficit > 8) and children without acidosis. Acidotic patients received fluid resuscitation with either Dextran 70 or Hetastarch at admission.

Results: Several markers of haemodynamic compromise were noted on admission including severe tachycardia, low stroke volume index (SVI) and high inferior vena cava collapsibility index (IVCCI) that improved with subsequent readings (fig 1). Overall, cardiac function assessed by ejection fraction (63.1% ± 5.2 vs. 71.9% ± 2.8 P=<0.001) and left myocardial performance index (LMPI) (0.32 ± 0.16 vs. 0.25 ± 0.08 P = 0.03) was mildly abnormal on admission compared to discharge. Acidotic patients had worse haemodynamic indicators, with significantly higher IVCCI on day 0 than non-acidotic patients (52.1 ± 21 .9 vs. 37.7 ± 15.4 P = 0.03); plus lower SVIs and worse cardiac function with higher LMPI (0.38 ± 0.18 vs. 0.26 ± 0.11 P = 0.05). SVI increased post first fluid bolus in 80% of acidotic children, from an average of 36.7 ml/m2 (95% CI: 30.9- 42.5) to 41.5 ml/m2 (95%CI: 37.19- 45.8, P = 0.007).

Inferior Vena Cava collapsibility Index in severe malaria by acidosis over time



Values =Mean ± SEM

Stages: 1=Day 0, 2= day 1, 3= discharge

P-values correspond to differences between day 0 and discharge using multi level analysis without categorizing by acidosis.

Inferior Vena Cava collapsibility Index in severe malaria by acidosis over time

Conclusion: Children with severe malaria have evidence of intravascular volume depletion and associated mild cardiac dysfunction which are more marked in those with metabolic acidosis. By optimizing cardiac output, this might aid microvascular flow and tissue perfusion with the aim of impacting on the metabolic derangement and associated high mortality in these children.

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29.014

Transfusion-associated Babesia infections: Reports received by the FDA 1997 to 2008

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Background: Babesiosis is a known transfusion-transmitted disease risk and there is no FDA-licensed test for donor screening. Approximately 80 transfusion-transmitted cases have been reported 1979 through 2008. This research evaluated the Babesia-related transfusion events reported to the Food and Drug Administration (FDA) with particular focus on numbers and characteristics of transfusion-related babesiosis fatality reports.

Methods: Data were collected from FDA's Blood Collection and Transfusion Fatality Reporting and Biological Product Deviation Reports (BPDRs) surveillance systems.

Results: From 2005 through 2008, the FDA received 10 transfusion-related babesiosis fatality reports after only one prior report in 1998. Recipients presented with symptoms 2.5 to 9 weeks after transfusion of implicated red blood cell units. In recent years, FDA has also seen an increase in Babesia-related BPDRs involving donors with post donation illness and reports of potential transfusion-transmitted disease. Most reports submitted to the FDA implicate B. microti.

Conclusion: The reports received by the FDA indicate Babesia infection may be a rare but increasing risk to the blood supply. Without a licensed screening test to prevent these transmissions, enhanced clinician awareness of the possibility of babesiosis in febrile, recent transfusion recipients may facilitate prompt diagnosis, improved prognosis, and more timely investigations to interdict infected units. Prompt reporting of babesiosis donor and transfusion-